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Differences in anxiety-related behavior and response to diazepam in BALB/cByJ and C57BL/6J strains of mice

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Abstract

The study in an ethological perspective of inbred strains of mice offers a more accurate description of anxiety-related behavior. In this context, behavioral performances of the BALB/cByJ and C57BL/6J strains were systematically compared in the staircase test, the light/dark test and the elevated plus maze test. A rarely used variable, the latency of the first entry in the dark box, was also recorded in the light/dark test. A new statistical approach to this measure meant that specific avoidance strategies could be differentiated in the two strains. A study of the behavioral responses of the two strains given treatment with diazepam, a widely used anxiolytic compound, was also carried out. Results showed substantial differences between BALB/cByJ and C57BL/6J strains. C57BL/6J mice had high baseline activity and exploration of a new environment, suggesting a low level of anxiety. BALB/cByJ mice displayed defensive and protective behavior, with limited exploration of the new environment together with low locomotor activity. The response to diazepam was also different for each strain: C57BL/6J mice showed higher sensitivity to diazepam treatment than did BALB/cByJ mice.

Keywords: Anxiety; Inbred strain of mice; Diazepam; Ethological measures; Light/dark; Elevated plus maze; Staircase

The extensive validation of animal models for anxiety in an ethological perspective [7,35] and the development of different statistical analyses [17,26,37] have produced improved descriptions of anxiety-related behavior in various strains of mice. However, it is difficult to compare the behavioral patterns of the strains using results from previous studies, mainly because of several experimental differences [22]. In such a context, a direct systematic comparison of inbred strains can be a useful method for drawing up strain-specific profiles. Two inbred strains of mice, BALB/cByJ and C57BL/6J, appeared to be of great interest for such studies as their emotional behavior has been reported as different or even opposite in the literature. BALB/cByJ is considered an emotive strain, whereas C57BL/6J is considered a non-emotive and active strain [13,27,34]. The aim of the present study was to make systematic comparisons and give accurate descriptions of behavioral performances by both strains, i.e. BALB/cByJ and C57BL/6J. Three different procedures, based on different ethological paradigms of anxiety in animals were used, taking several variables into account.

The staircase test is based on the inherent tendency of rodents to explore a novel environment. It gives a general idea on the level of emotivity of the mice. Several authors [41,43] have assumed that the number of steps climbed (NSC) reflects the locomotor component of mouse behavior whereas the number of rearings (NR) reflects the exploration component. Both of these components are influenced by the level of anxiety in the animal. The strains used in most studies are NMRI [32,42] and CD1 [31,33,41]. Neither BALB/cByJ nor C57BL/6J mice had been used for this very simple test.

The light/dark situation is based on a conflict between the natural tendency of mice to explore a new environment and the avoidance of brightly lit space [14]. Exploration is assessed by the number of transitions (NTR) between a lit box directly adjoining a dark box. Avoidance of the brightly

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lit space can be assessed by the time spent in the dark box (TDB). Only one study showed BALB/cByJ to have little locomotor activity and a low baseline exploratory tendency, unlike C57BL/6J [13]. Another comparison of the two strains has been made on small groups of mice [5] using an apparatus different from the one commonly used in the literature: a tunnel was placed between the two boxes. The study observed that most of the BALB/cByJ mice spent more time in the tunnel than in the other boxes. By staying in the tunnel, the mice could avoid exploring the new environment and therefore, the number of transitions cannot be compared with the figures in other studies. In the present study, an apparatus without a tunnel was used so as to make the animal choose between the two boxes. A third variable, latency of the first entry into the dark box (LAT), was also recorded. This variable has rarely been used, despite the fact it is recognized as a measure of aversion to the bright box [4,11]. In addition, an original statistical approach, a lifetest procedure, was used to give an accurate description of the variation of latency time for each mouse population. Using the statistical treatment of the latency measure, specific avoidance strategies could be differentiated.

The elevated plus maze is based on the conflict between exploration and avoidance of a novel environment set in a void and produces several ethological measures [37], which can produce a profile for anxiety in rodents. Both enclosed and open arms in the apparatus generate exploration (as assessed by the number of entries in both types of arms and rearings) and risk assessment (as assessed by the number of head-dippings and stretched attend postures). Both these behavioral components are known to be sensitive to anxiety [1]. Avoidance of open arms and exploration of the extremities of the open arms also seem to indicate the level of anxiety. The test has been validated in mice [26] and should differentiate emotional responses in relation to the strains. In the study by Trullas and Skolnick [45], BALB/cByJ mice were, surprisingly, more active than C57BL/6J mice in the elevated plus maze test, but in an open-field test, BALB/cByJ clearly did not explore the apparatus, although C57BL/6J did. These contradictory results showed the need for further comparisons of the two strains using behavioral measures in addition to the level of activity.

The comparison was completed by investigating the response of BALB/cByJ and C57BL/6J mice to an anxiolytic treatment (with diazepam). Previous studies of rodents using the staircase test have shown that diazepam causes a decrease in the number of rearings at certain doses, although it does not change or increase the number of steps climbed. The authors therefore suggested that the number of steps climbed was more a reflection of the locomotor component, while the number of rearings reflected the anxiety level in the rats or mice [42,43]. No data relating to this were available on BALB/cByJ and C57BL/6J.

In the light/dark test, the anxiolytic effect on mice of diazepam, as well as other anxiolytic compounds, has been shown to cause an increase in the number of transitions between the two boxes [12,15], although a sedative effect was observed at high doses (5 mg/kg). In C57BL/6J mice, an increase in the number of transitions and an increase in the time spent in the lit box were reported by Costall et al. [11]. BALB/cByJ mice presented the same diazepam-induced behavioral responses as C57BL/6J mice [2,20]. Very few data are available on the latency to enter the dark box. One study [21] of the C57BL/6J strain only, showed a dose-dependent increase in the latency of the first entry into the dark box.

The elevated plus maze can also detect responses to anxiolytic and anxiogenic drugs [30]. It is known that diazepam has an anxiolytic action in both rats and mice, affecting several variables [29,36,38]; but most of the studies on the behavioral responses of mice to diazepam are of the DBA2/J strain [9,16,38]. An investigation of both BALB/cByJ and C57BL/6J strains was therefore needed using the three different tests.

1. Methods

1.1. Animals

Two inbred strains of mice were used: BALB/cByJ and C57BL/6J (IFFA Credo, France). Three-month-old males were housed under standard conditions of 22°C, a 12:12 h photoperiod with lights on at 0800 h, water and food available ad libitum, and dust-free soft wood sawdust bedding. The experiments reported herein were performed in compliance with the ethical guidelines of the French Ministry of Agriculture. Table 1 shows the distribution of the mice according to each test and each dose of diazepam used in the study.

1.2. Behavioral procedures

To facilitate adaptation, the animals were moved to the experimental room 1 h before each test session.

Table 1
Number of mice in experimental groups

Strains	Diazepam dose (mg/kg)	Tests		
		Staircase	Light/dark	Elevated plus maze
BALB/cByJ	0	44	52	15
	0.5	18	25	18
	1	24	23	19
	2	17	22	20
	4	14	0	0
C57BL/6J	0	44	43	18
	0.5	19	23	17
	1	20	23	20
	2	19	23	19
	4	15	0	0

Different animals were used for the different tests and the different doses of diazepam.

1.2.1. Staircase test

The wooden apparatus consisted of five steps enclosed by a 12.5-cm-high wall. Mice were placed individually on the floor of the box facing away from the steps. Two behavioral parameters were recorded: NSC and NR. A step was considered climbed when the four paws of the mouse were placed on it. The duration of the test was 3 min. The apparatus was cleaned with a moist sponge between each trial.

1.2.2. Light/dark test

The experimental room was lit by a single dim red light. The apparatus consisted in two polyvinyl plastic boxes ($20 \times 20 \times 14$ cm), one dark and covered by a dark, opaque top, the other bright with a 100-W light 20 cm above the floor of the box. The mice could move from one box to the other through an open door between the two boxes. At the beginning of the test, the mice were placed in the illuminated box, facing the entrance to the dark box. The test started when the animal entered the dark box for the first time. The latency (LAT) of the first entry was recorded. NTR between the two boxes and the TDB were also recorded over a 5-minn period. The apparatus was thoroughly cleaned between tests.

1.2.3. Elevated plus maze

The apparatus was made of dark polyvinyl plastic. The maze was elevated to a height of 50 cm with two open (30×8.5 cm) and two enclosed arms ($30 \times 8.5 \times 17.5$ cm), arranged so that the arms of the same type were opposite each other, connected by an open central area. Experiments were performed with the same lighting as in the rearing facility. At the beginning of the experiment, each mouse was placed individually in the center of the maze, facing one of the open arms and observed for 5 min. Different measures were recorded:

(a) Number of open-arm entries (an arm entry was defined as all four paws inside the arm)
(b) Number of total arms entries
(c) Percentage of open-arm entries (number of open-arm entries/total number of arms entries $\times$ 100)
(d) Percentage of time spent in the open arms (time in open arms/total time in arms $\times$ 100)
(e) Number of rearings
(f) Number of stretched attend postures
(g) Number of head-dippings
(h) Number of open-arm ends visited

The apparatus was cleaned thoroughly between tests. Test sessions were recorded using a vertically mounted video camera. The tapes were scored by a trained observer blind to treatment conditions.

1.3. Pharmacological treatment

The treated mice received diazepam (Hoffmann-La Roche, Basel) dissolved in physiological saline with two drops of Tween 80. Mice in the control group received the vehicle (physiological saline and Tween). Injections were given 20 min before the test, in a volume of 1 ml/200 g body weight. Since no significant difference was found in former studies between animals receiving vehicle injection and animals with no injection at all, this last control was not included here.

1.4. Statistical analysis

For each test, the statistical analysis was the same: an analysis of covariance, using the mixed procedure of the SAS software, was first carried out for the factor «strain» and the covariance factor «dose» for each variable. In order to respect assumptions of this linear analysis, we looked for the best tranformation of the data recorded for each variable. Such a transformation is generally carried out in order to linearise relationships, stabilise variances or reduce skewness. For each variable, which needed to be transformed, the SAS-LAB software [39] has used a maximum likelihood analysis to find the best transformation. We obtained the estimation of linear regression of response on dose or quadratic regression of response on dose for each strain. The estimation of the strain $\times$ dose interaction was used to compare parameters of the regressions, using an F test. The mean values of each variable were then systematically compared strain to strain for each dose by a Student's equal t test.

1.4.1. Staircase test

No transformation of the data was carried out before statistical analysis. Regression dose–response curves were obtained for both the NSC and the NR.

1.4.2. Light/dark test

LAT and NTR were analyzed after log10 transformation ($x_t = \log10(x)$). TDB was analyzed after a 1.5 exponent transformation ($x_t = x^{1.5}$). After regression analysis, dose–response lines were obtained for NTR and LAT. A dose–response curve was obtained for TDB.

For the LAT, a lifetest procedure was used as a significant number of mice reached maximal latency (180 s) without entering the dark box. The objective was to ensure that these mice were included and to find an analysis that could help interpret the situation. An SAS lifetest procedure was used for survival data analysis. The maximal latency was the right-censored value (180 s) of the analysis. For each strain and each dose, a population–time curve showed the percentage of mice crossing into the dark box as time passed. A Log-Rank test (nonparametric test) was carried out to test the significance of interstrain differences. The censored data (latency = 180 s) were taken into account in this analysis. A risk ratio, determined by a Cox regression for each strain, reflected the probability of a change in the latency when there was a change in the treatment dose.

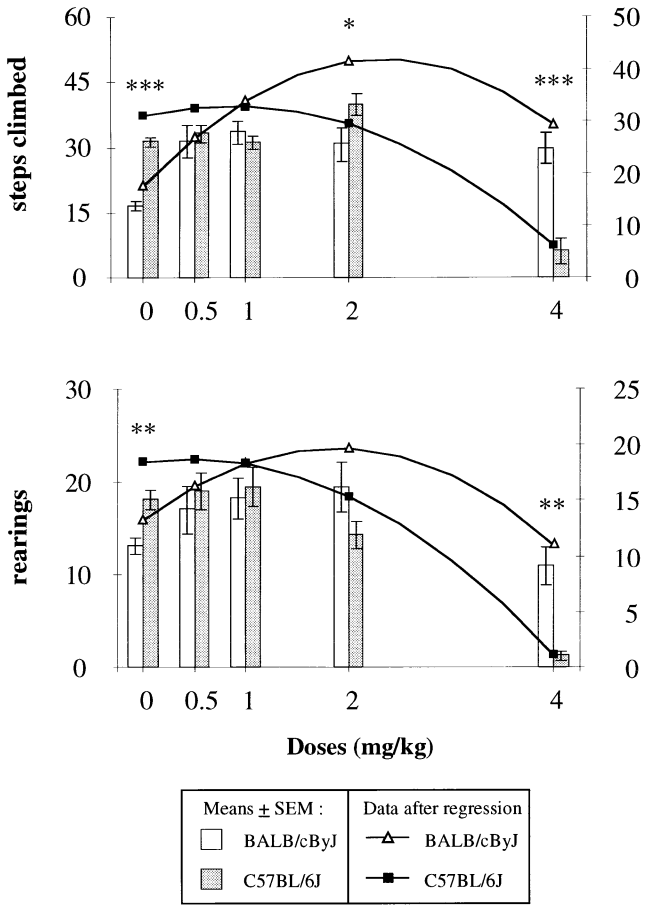

Fig. 1. Staircase test. Means (±S.E.M.) of steps climbed and means of rearings after injection of vehicle (dose 0) or diazepam (0.5, 1, 2 and 4 mg/kg) for BALB/cByJ and C57BL/6J mice. Data shown after regression analysis without previous transformation. Statistically significantly different for the two strains: * $P<.05$, ** $P<.001$, *** $P<.0001$, Student's t test.

1.4.3. Elevated plus maze

A squared transformation was made for the percentage of open arms visited, the number of rearings, number of open-arm ends visited and the percentage of time in open arms. The number of stretched attend postures and head-dippings were analyzed after log10 transformation. Regression dose–response lines were obtained for the percentage of open arms visited, the mean of open arms visited, the percentage of time in open arm, total arms visited, the number of rearings and the number of visits to the end of the open arms. Regression dose–response curves were obtained for the number of stretched attend postures and head-dippings.

2. Results

2.1. Staircase test

The analysis of covariance showed significant differences between BALB/cByJ and C57BL/6J for NSC [$F(1,222)=39.71$, $P<.0001$] and for NR [$F(1,222)=10.27$, $P<.002$]. Treatment had an effect on NSC [$F(1,222)=49.50$, $P<.0001$] and on NR [$F(1,222)=9.35$, $P<.003$] for both strains. There was also a dose × strain interaction for NSC [$F(1,222)=20.67$, $P<.0001$] and NR [$F(1,222)=4.46$, $P<.05$].

A strain-to-strain comparison showed significant differences between BALB/cByJ and C57BL/6J control groups for both variables: C57BL/6J climbed more steps and reared more often than BALB/cByJ (Fig. 1). For the BALB/cByJ mice, diazepam treatment increased both NSC and NR for low doses (0.5 and 1 mg/kg): at the 2 mg/kg dose, NSC increased while NR stopped increasing. A decrease in both variables was then observed for the 4-mg/kg dose. A slight shift between the quadratic curves of NR and NSC was also observed for C57BL/6J. At the 1-mg/kg diazepam dose, NR decreased while NSC kept on increasing. At 2 mg/kg, NR decreased while NSC did not change. At 4 mg/kg, the two variables decreased, most probably because of the myor-

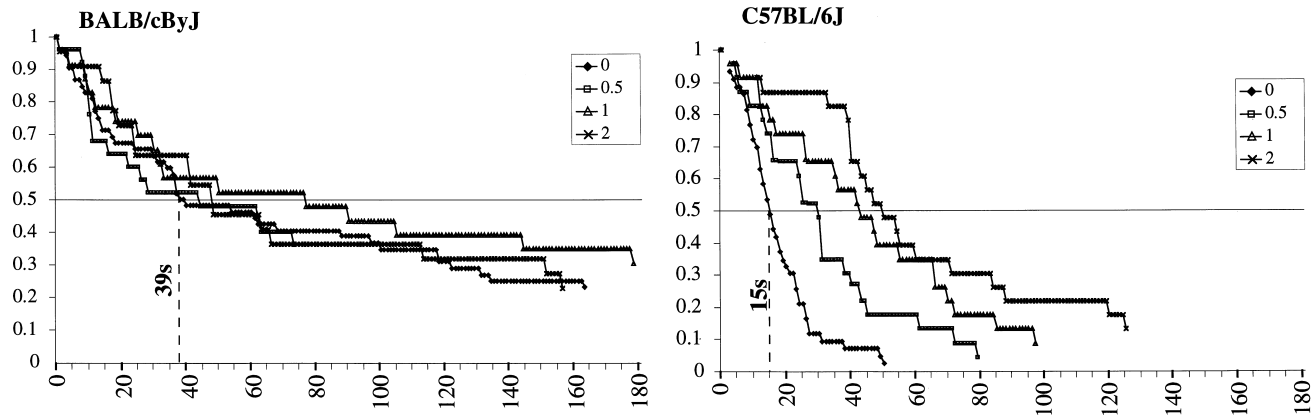

Fig. 2. Light/dark test. Population–time curve showing the percentage of mice crossing into the dark box for BALB/cByJ and C57BL/6J mice after injection of vehicle (dose 0) or diazepam (0.5, 1 and 2 mg/kg). Right-censored value is 180 s, i.e. the maximal latency recorded in the experiment.

elaxant effect of diazepam. The latter results concur with the previous findings in other strains and species of rodents [43]. It may be noted that the decrease in NSC and NR under the myorelaxant effect of diazepam occurred earlier for the C57BL/6J strain than for BALB/cByJ.

2.2. Light/dark test

2.2.1. Latency

2.2.1.1. Control group. Comparison of the two strains showed significant difference for latency (Log-Rank χ^2

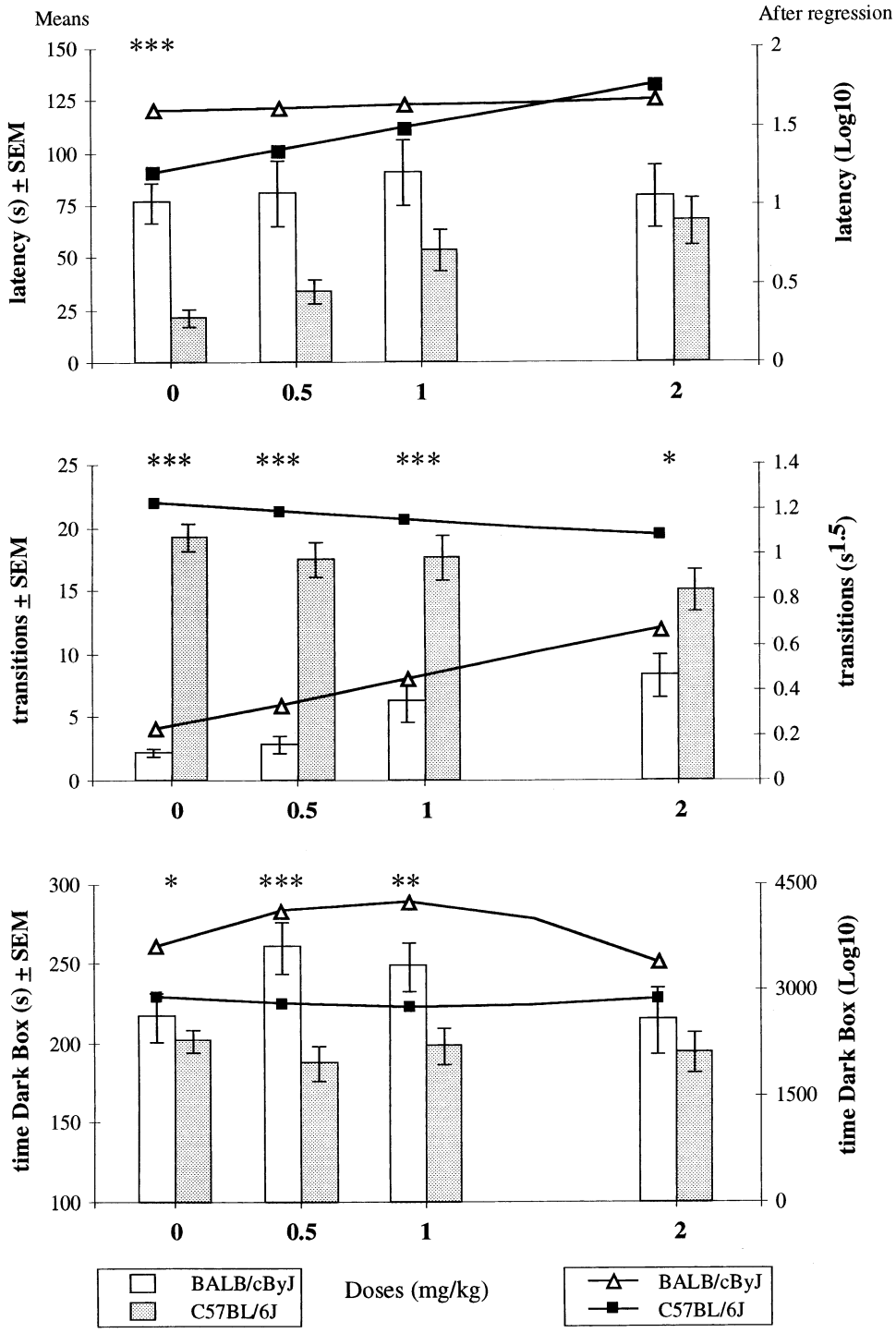

Fig. 3. Light/dark test. Values of LAT of the first entry into the dark box, NTR and TDB for BALB/cByJ and C57BL/6J mice after injection of vehicle or diazepam (0, 0.5, 1 and 2 mg/kg). Means are represented by bar charts with error bars (left *y*-axis). Data after regression analysis are represented by lines or curves (right *y*-axis). Statistically significantly different for the two strains: * $P<.05$, ** $P<.001$, *** $P<.0001$, Student's *t* test.

= 57.40, *df* = 7, *P* < .0001). For the BALB/cByJ population (Fig. 2), the lifetest procedure distinguished two subgroups: the first 50% of the mice had short latency of entry into the dark box (39 s), whereas the other 50% stayed longer before leaving. 23% of BALB/cByJ mice did not leave the lit box by themselves: at the censored value of 180 s, they were gently pushed into the dark box. The profile of the survival curve was completely different for C57BL/6J (Fig. 2). The first 50% of the mice left the lit box within 15 s. By 50 s, all the mice except one had left the lit box.

2.2.1.2. Effects of diazepam. A classical strain-to-strain comparison with Student's equal *t* test did not show any significant differences between the two strains for latency (Fig. 3). The Log-Rank test analysis showed that diazepam treatment affected performances in both strains (Log-Rank $\chi^2 = 15.81$; $P < .0001$), but the effect

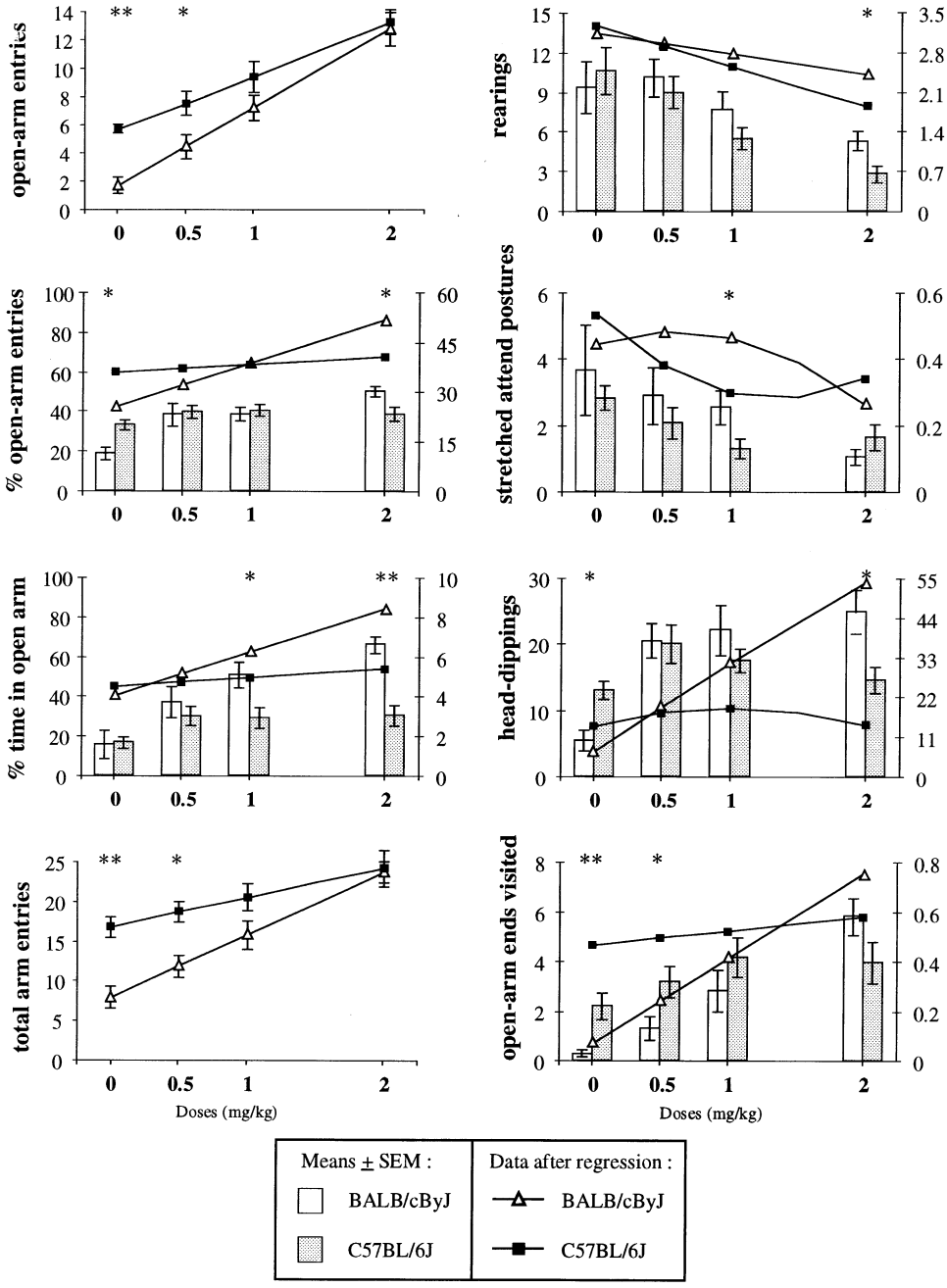

Fig. 4. Elevated plus maze. Values of the different behavioral measures recorded for BALB/cByJ and C57BL/6J mice after injection of vehicle or diazepam (0, 0.5, 1 and 2 mg/kg). Means are represented by bar charts with error bars (left *y*-axis). Data after regression analysis are represented by lines or curves (right *y*-axis). Statistically significantly different for the two strains: * $P < 0.05$, ** $P < .001$, *** $P < .0001$, Student's *t* test.

was different for each strain (Log-Rank $\chi^2 = 22.34$; $P < .0001$).

In BALB/cByJ mice (Fig. 2), there was a significant decrease in latency at a dose of 0.5 mg/kg: 50% of the mice entered the dark box quickly (latency < 30 s). Higher doses of diazepam treatment had no effect on latency. This explains the high risk ratio (RR = 0.959; $P = .775$) found for the BALB/cByJ strain. The percentage of mice not leaving the lit box voluntarily ranged from 22% to 30%.

The latency for C57BL/6J mice (Fig. 2) increased (80 to 125 s) with the dose of diazepam and the risk ratio was therefore low (RR = 0.467; $P < .0001$). There was also a slight increase in the number of diazepam-treated mice that did not leave the lit box (1–12%), but even at a high dose (2 mg/kg), most C57BL/6J mice left the lit box.

2.2.2. Transitions

Overall, significant differences were observed between the two strains [$F(1,230) = 249.66$, $P < .0001$]: BALB/cByJ mice made very few transitions, whereas C57BL/6J made many transitions between the two boxes. There was a dose-related effect on NTR [$F(1,230) = 5.82$, $P < .02$] for both strains: higher doses of diazepam caused a slight decrease in NTR for C57BL/6J, but an increase in NTR for BALB/cByJ (Fig. 3).

2.2.3. Time spent in the dark box

Overall, BALB/cByJ mice spent significantly more time in the dark box than C57BL/6J [$F(1,228) = 5.11$, $P < .03$]. A dose-related effect of diazepam was observed but only for BALB/cByJ (Fig. 3): an increase in TDB at 0.5 and 1 mg/kg and a decrease in TDB for 2 mg/kg. The quadratic regression curve was different for the C57BL/6J strain: TDB did not change under diazepam treatment (Fig. 3). Comparison strain to strain showed significant differences for all doses, except for the 2-mg/kg dose, which correlated with a substantial decrease in TDB for BALB/cByJ.

2.3. Elevated plus maze

2.3.1. Control group

Compared to C57BL/6J, the BALB/cByJ mice recorded significantly fewer total arm entries ($t = -4.94$, $P < .0001$) and open-arm entries ($t = 5.93$, $P < .0001$), and a lower percentage of open-arm entries ($t = 3.66$, $P < .0010$) and head-dippings ($t = 3.68$, $P < .001$). They also visited fewer open-arm ends ($t = 3.47$, $P < .003$). There was no significant difference between the two strains for the percentage of time spent in the open arms, the number of rearings or the number of stretched attend postures.

2.3.2. Effects of diazepam

Diazepam treatment affected all behavioral measures, for both strains: dose-dependent increases were observed for the number of open-arm entries [$F(1,139) = 9.46$, $P < .003$], the total number of arms visited [$F(1,139) = 14.56$, $P < .0002$], the percentage of open-arm entries [$F(1,137) = 20.52$, $P < .0001$], the percentage of time spent in the open arms [$F(1,140) = 30.13$, $P < .0001$], the number of head-dippings [$F(1,139) = 19.51$, $P < .0001$] and the number of open-arm ends visited [$F(1,141) = 28.24$, $P < .0001$]. However, decreases were recorded in the number of rearings [$F(1,141) = 26.35$, $P < .0001$] and the number of stretched attend postures [$F(1,141) = 8.48$, $P < .005$] (Fig. 4). The decrease in the number of rearings was also dose-dependent, whereas the decrease in the number of head-dippings occurred at different doses for each strain. At a low dose of diazepam (0.5 mg/kg), the number of stretched attend postures decreased for C57BL/6J, while it increased slightly for BALB/cByJ. The number of stretched attend postures then decreased from the point reached in each strain. Diazepam treatment also increased the number of head-dippings but the effect was greater on BALB/cByJ. Comparison of the behavioral response to diazepam shows significant differences between the two strains, the greatest contrast being at the maximum dose of 2 mg/kg.

3. Discussion

The aim of the present study was to investigate anxiety-related behavioral differences between the BALB/cByJ and C57BL/6J strains in different tests. According to Montgomery [28], exposure to a novel environment can induce both exploratory and fear drives, thus generating an approach–avoidance conflict behavior. Ethological measures linked to the exploratory component of murine behavior were therefore analyzed first.

3.1. Exploration, locomotion and vertical activity

In the staircase test, BALB/cByJ control mice climbed significantly fewer steps and did fewer rearings than C57BL/6J controls. In the light/dark procedure, BALB/cByJ made very few transitions between the two boxes [3,13,20] compared to C57BL/6J. In the elevated plus maze, C57BL/6J mice recorded more rearings (considered as vertical activity), head-dippings and total arms entries than BALB/cByJ. These interstrain differences in general activity observed in the three tests cannot be explained solely by differences in locomotor activity. BALB/cByJ mice have been shown to have a higher daily locomotor activity than C57BL/6J [24,25]. According to Archer [1], inhibition of exploratory behavior is commonly associated with high emotionality or anxiety. As general activity and locomotion are difficult to dissociate from exploration, both types of behavior had to be considered. Taken together, the inhibition of locomotor behavior and the low level of exploration of

BALB/cByJ mice suggest that the strain is more emotional or anxious than for C57BL/6J.

The acute effect of different doses of diazepam confirms these results. Anxiolytic treatment, even at a low dose, caused an alteration of both locomotor and exploratory measures in BALB/cByJ: NSC, NTR, total arms entries, open-arm entries and number of head-dippings increased in treated mice. The strain displayed exploratory behavior in all new environments encountered in the experiment. The anxiolytic-induced increase in C57BL/6J exploration suggests that mice in the control group were also subject to anxiety, at a low level, in the same test situations.

3.2. «Pure» anxiety

Observations of other behavioral measures in the light/dark and elevated plus maze tests confirm the difference in anxiety-related behavior between BALB/cByJ and C57BL/6J. BALB/cByJ control mice spent more time in the dark box than C57BL/6J controls, which concurs with other observations of the strain avoiding bright environments [19]. Diazepam decreased the time spent by C57BL/6J in the dark box, thus increasing the exploration of the strain, which already had a high exploratory baseline. Only high doses of diazepam decreased TDB by BALB/cByJ, whereas NTR increased, even at a low dose. This suggests that TDB is a stronger index of anxiety, whereas NTR reflects both anxiety and exploration.

In the elevated plus maze, both strains clearly preferred closed arms to open arms; this observation concurs with findings of studies of several inbred strains of mice [18,23,26,36]. However, the present study did observe striking differences between the two strains. Observations of open-arm behavior tally with those in the study by Conti et al. [10] but contradict the findings of Cole et al. [8] and Trullas et al. [44,45]. Cole et al. [8] found that BALB/cByJ displayed a high level of baseline activity in the open arms, which is very unusual for the strain. Trullas et al. [44,45] observed that BALB/cByJ showed less avoidance of the open arms and were less reactive to open arms than C57BL/6J. This difference may be due to the fact that the test was not conducted under dim red light although the brightness was the same as in the animal facility so as to avoid any possible total behavioral inhibition. BALB/cByJ mice visited fewer arms (both open and closed) and open-arm ends, entered fewer open arms, spent less time in open arms and did fewer head-dippings than C57BL/6J. The dose-dependent increase of all these measures for diazepam-treated BALB/cByJ argues in favor of the hypothesis that they provide a good assessment of the anxiety level in the strain. Interestingly, C57BL/6J mice presented a different response to diazepam: the same behavioral measures (total arm entries, open-arm entries, time spent in open arms, open-arm ends visited and head-dippings) did not alter, or registered only a slight increase. The difference in the behavioral response to diazepam is consistent with the difference in emotionality between the two strains as observed in other tasks.

3.3. Risk assessment

According to Blanchard et al. [6], risk assessment is a central element in the behavioral expression of anxiety. More recent studies of murine behavior in the elevated plus maze [37,40], have shown that the stretched attend posture could well reflect the risk assessment process. In the present study, BALB/cByJ recorded more stretched attend postures but fewer head-dippings than C57BL/6J. The observation appears to coincide with the distinction made in the factorial analysis by Rodgers and Johnson [37]: stretched attend posture loaded for risk assessment, whereas head-dippings loaded mainly for exploration. Furthermore, in BALB/cByJ, the number of stretched attend postures, unlike the other measures, only altered with high doses of diazepam, suggesting that risk assessment is more pronounced than other anxiety-related behavior patterns. The less anxious C57BL/6J recorded fewer stretched attend postures at a dose ≥ 0.5 mg/kg diazepam, whereas they did more head-dippings at the same dose. Stretched attend postures occurred more often when moving from the closed arms to the open arms, whereas head-dippings were generally directed to the empty space around the apparatus. Unlike head-dippings, the stretched attend posture appears to be defensive rather than approach behavior. In conclusion, these findings suggest a difference in the risk assessment strategies used by the BALB/cByJ and C57BL/6J strains.

3.4. Decision-making

Decision-making is closely related to risk assessment, which consists in gathering information on the environment and its potential dangers, and involving varying degrees of caution when doing so. Decision-making leading to passive avoidance or flight occurs after such risk assessment. In the present study, the light/dark procedure appears to be of particular help in evaluating decision-making, given the clear interstrain differences observed in the latency of entering the dark box. C57BL/6J mice started by exploring the lit box extensively before quickly leaving the lit box for the dark one. It may be assumed that this movement reflects decision-making, which is swift, but not instantaneous. The move cannot be considered simply as flight, as it was followed by intensive exploration of the entire apparatus and a high NTR. In fact, the diazepam treatment did not change the TDB and only caused a very slight decrease in exploration showing that C57BL/6J mice had a low level of anxiety in this situation.

Among the BALB/cByJ strain, the lifetest analysis distinguished two types of response. Half the control group had short latency but, unlike C57BL/6J, the short latency was followed by an extended length of time in the dark box and very few transitions. It may be assumed that the mice fled the brightness of the box. The other half of the BALB/cByJ control group had long latency with inhibition of locomotor and exploratory activities, followed by an extended stay in the dark box and very few transitions. BALB/cByJ mice showed two types of avoidance response (active vs. passive avoidance), which correspond to two different latencies. These results suggest intrastrain differences in decision-making time for BALB/cByJ mice, involving two possible avoidance responses (flight vs. immobility).

The use of two different genetically controlled strains of mice revealed substantial differences in behavioral response to experimental anxiogenic situations. Highly emotional BALB/cByJ mice are distinguished by a defensive and protective behavior, with limited exploration of new environment, together with low locomotor activity. Extended risk assessment leads to either flight or passive avoidance. On the opposite, low-emotional C57BL/6J mice have a high baseline activity (vertical activity and locomotion) when placed in a novel environment and explore to gather as much information as possible. Swift risk assessment and decision-making lead to less avoidance in C57BL/6J than in BALB/cByJ. Finally, the results also showed that the anxiolytic effect of diazepam on the behavior always occurred earlier in C57BL/6J than in BALB/cByJ.

As shown in the present study, anxiety is a complex phenomenon expressed by several behavioral components and the systematic use of different ethological measures could be a major contribution to the behavioral and pharmacological understanding of anxiety mechanisms.

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